Interpreting In Vivo Effects of Thyroid Synthesis Inhibitors Through the Lens of Ex Vivo Assays

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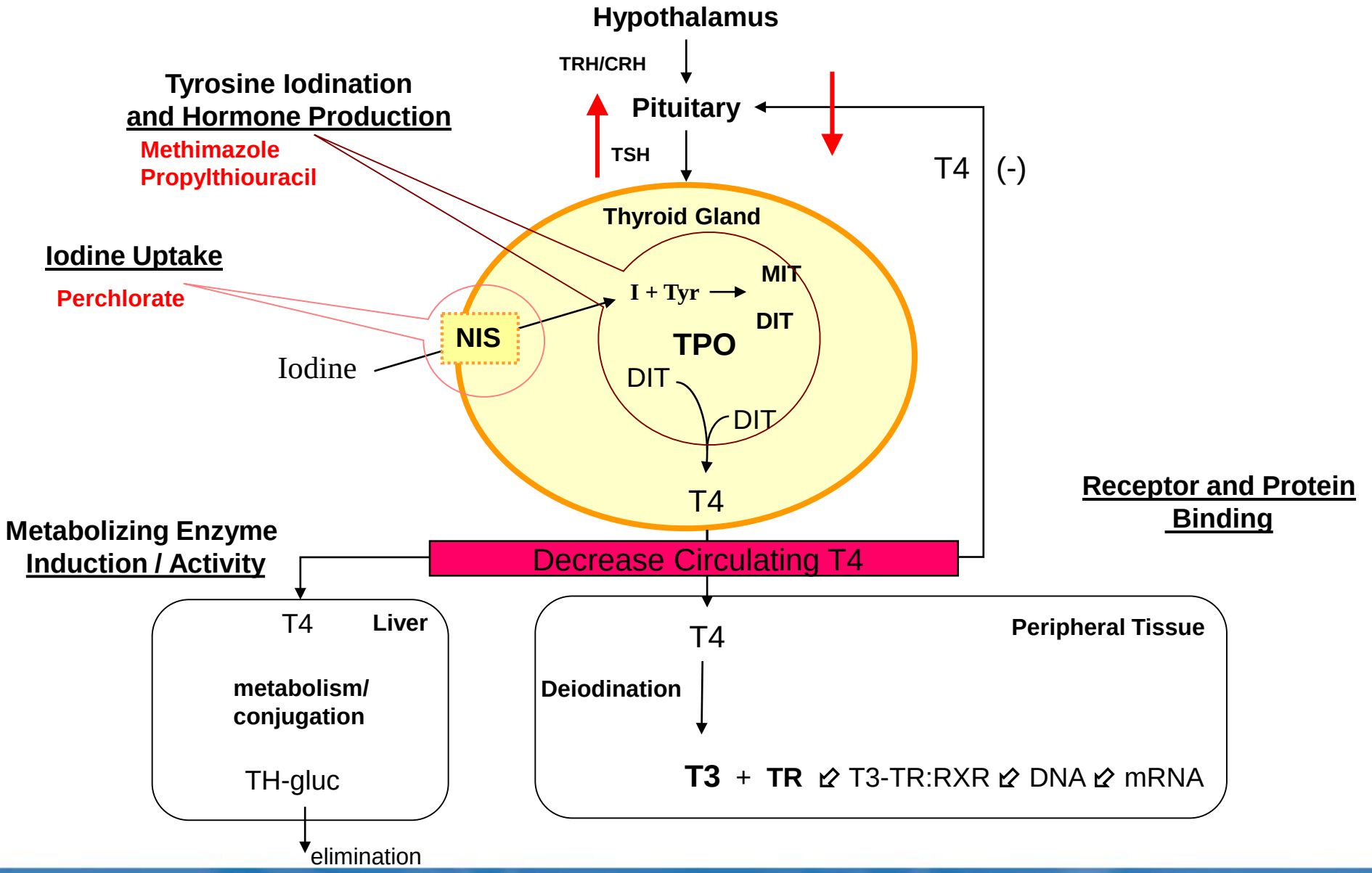
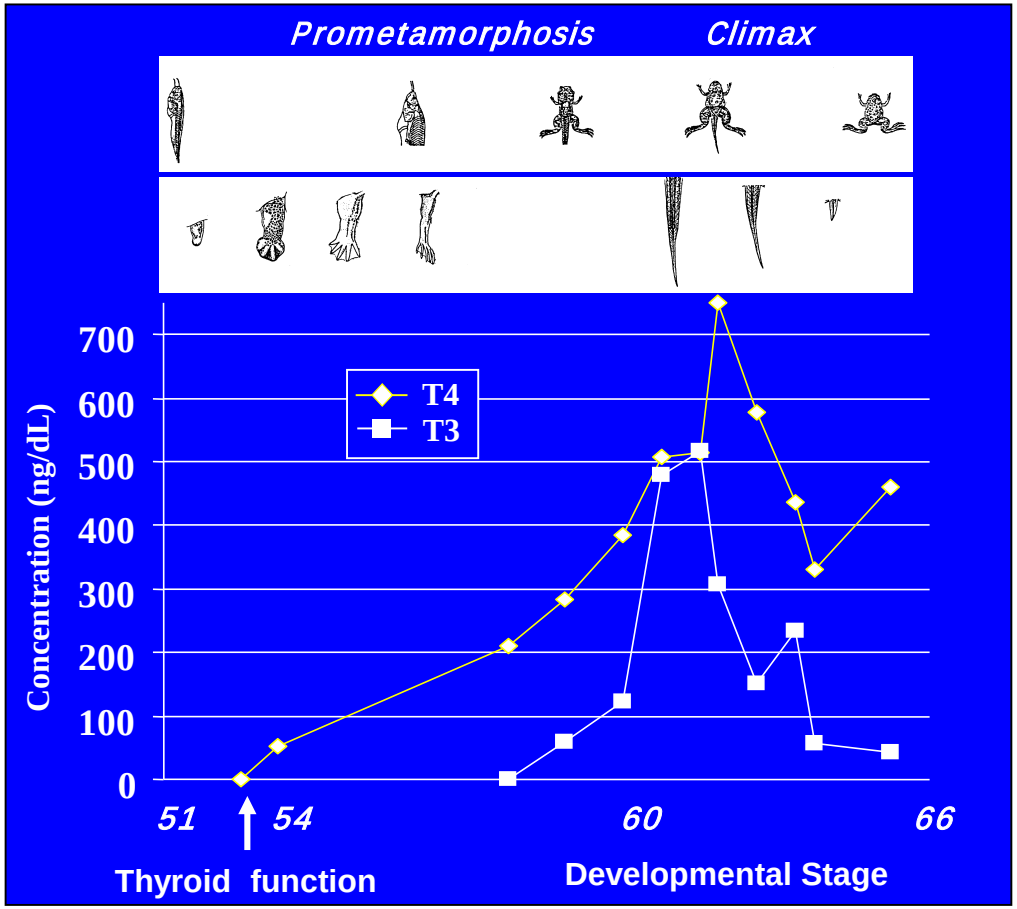
Potential Endpoints for Thyroid Hormone Disruption



Amphibian Metamorphosis Assay



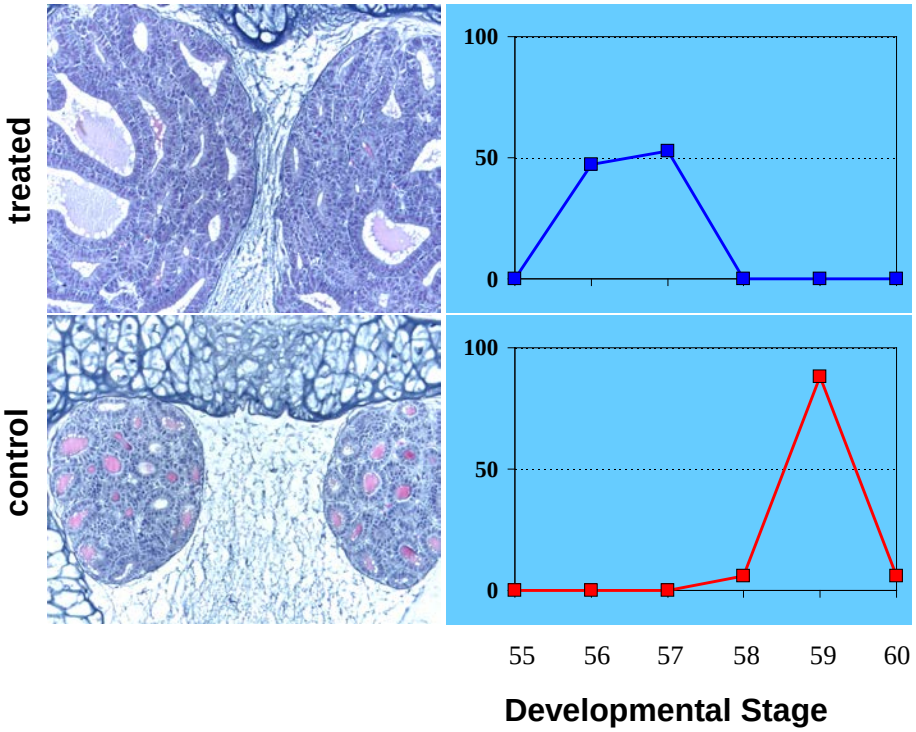
Metamorphosis is a Thyroid Hormone-Dependent Process

Amphibian Metamorphosis Assay

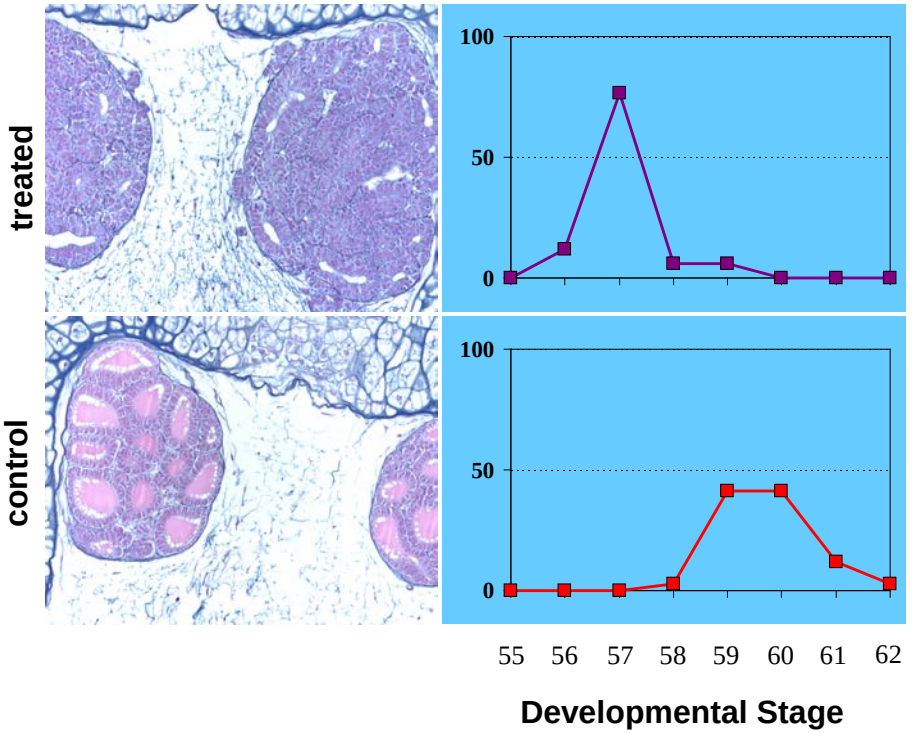
- Initiated with tadpoles at onset of thyroid function
- 14-21 days exposure
- Apical Endpoints
  - Developmental stage
  - Thyroid histology

Amphibian Metamorphosis Assay

Methimazole

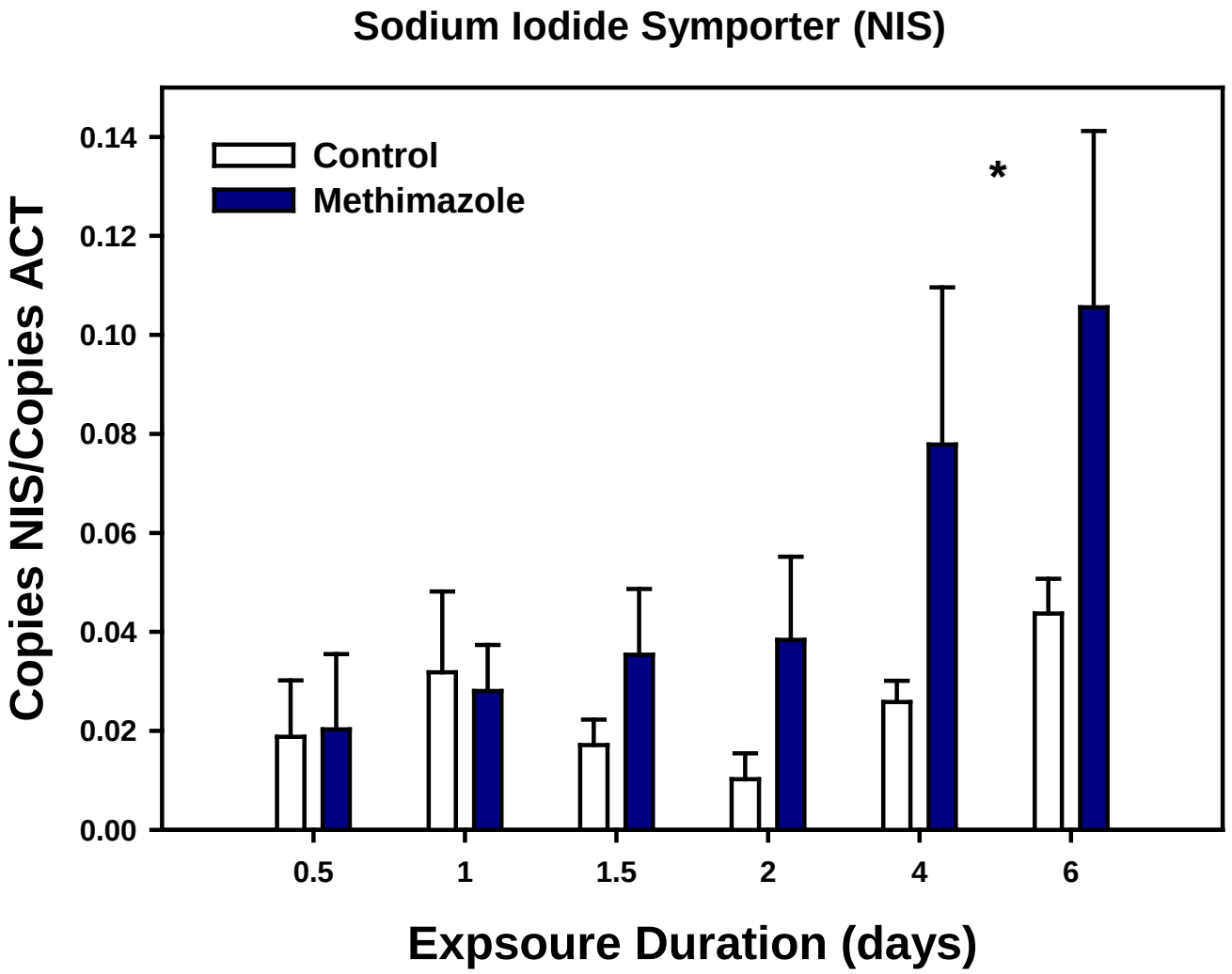


Perchlorate

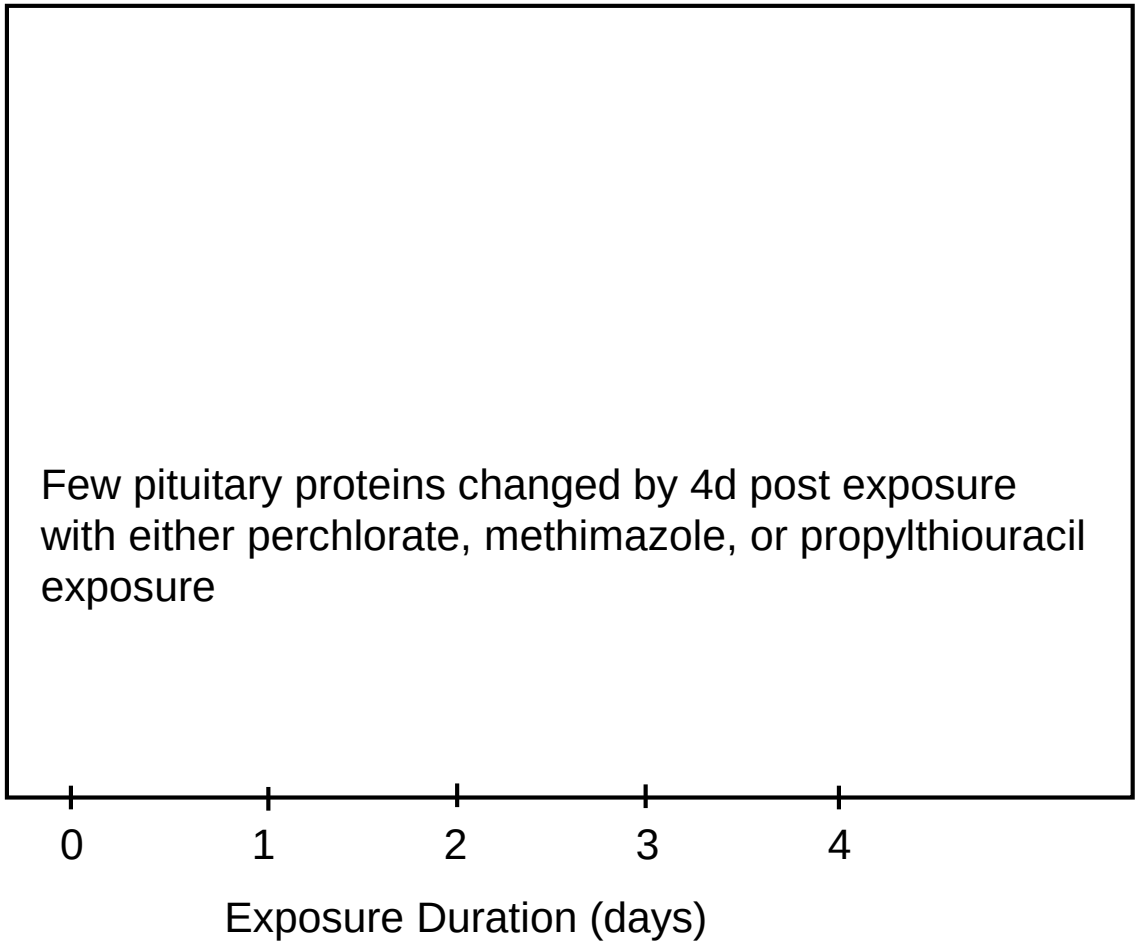


Are there effects that occur earlier than changes in histology or development that can be used as diagnostic indicators of thyroid disruption ?

In Vivo Gene Expression Following Exposure



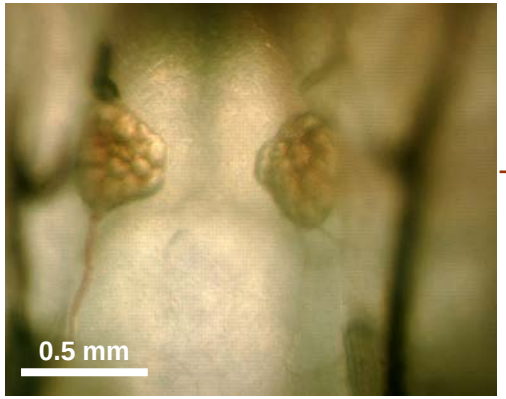
Pituitary Proteins Following Exposure



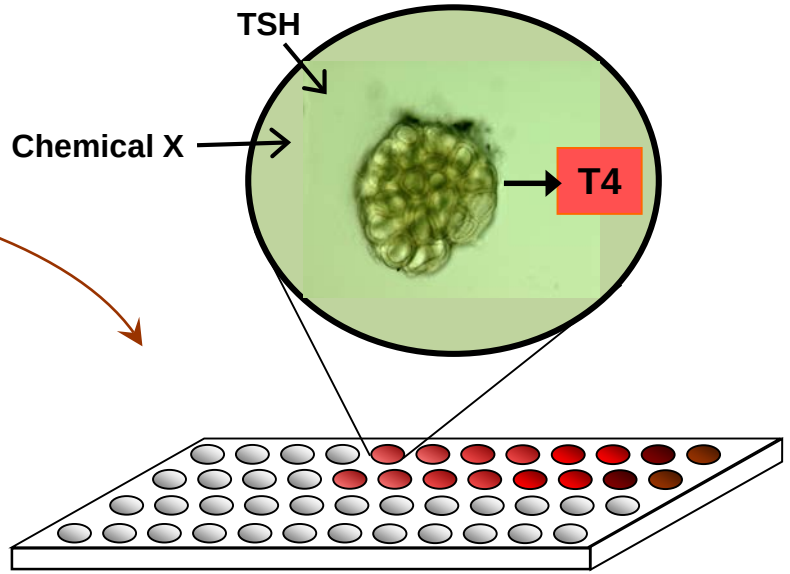
Thyroid Gland Explant Cultures

Dissect thyroid glands from pro-metamorphic tadpoles

- Culture in 96-well plates in L-15 media (BSA, antibiotic, 1 µM KI)
- Stimulate with TSH and treat with graded concentrations of test chemical
- Measure T4 released to media by RIA
- Measure TSH responsive gene expression by QPCR.

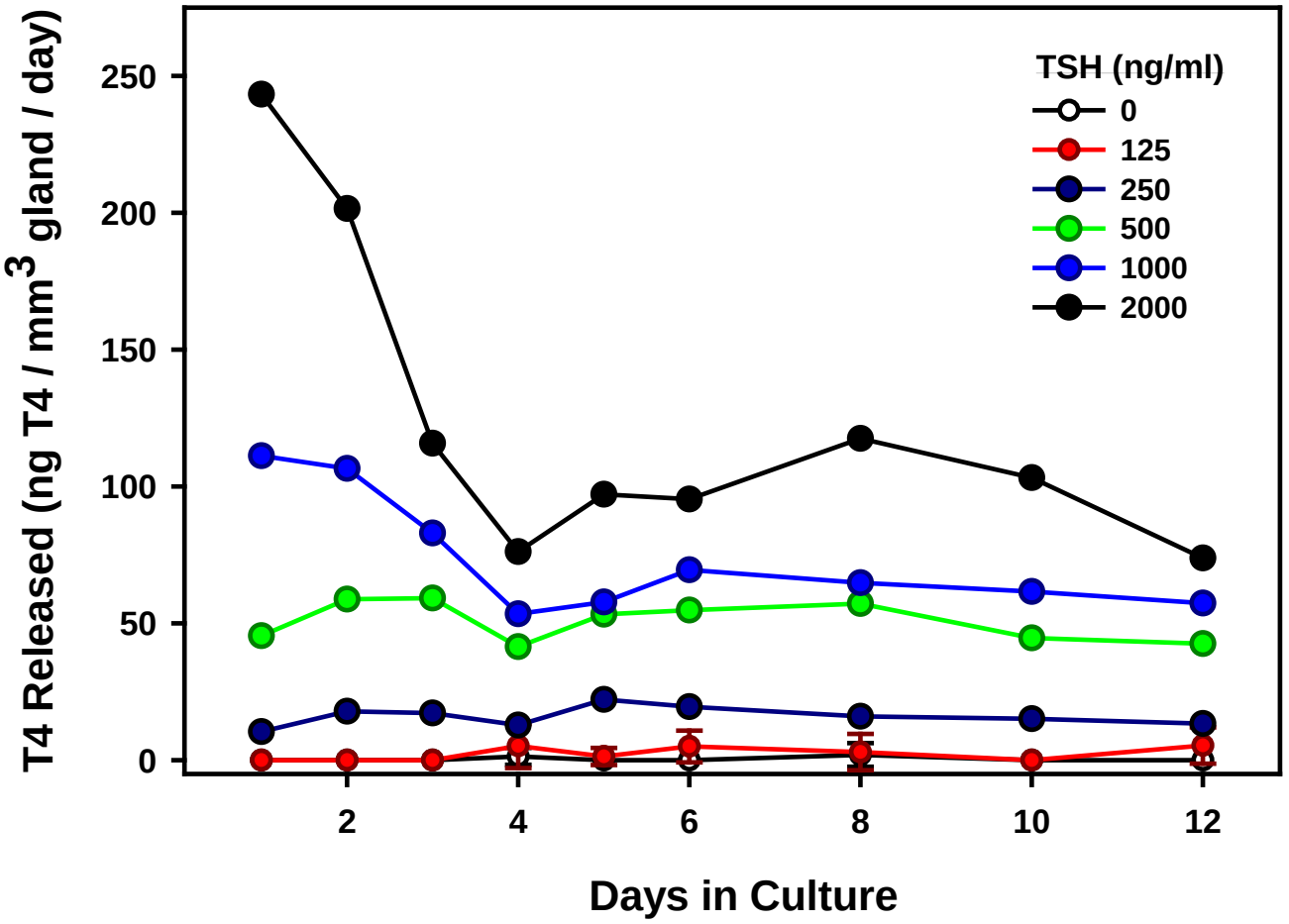


Paired thyroid glands in NF stage 59 *X. laevis* tadpole



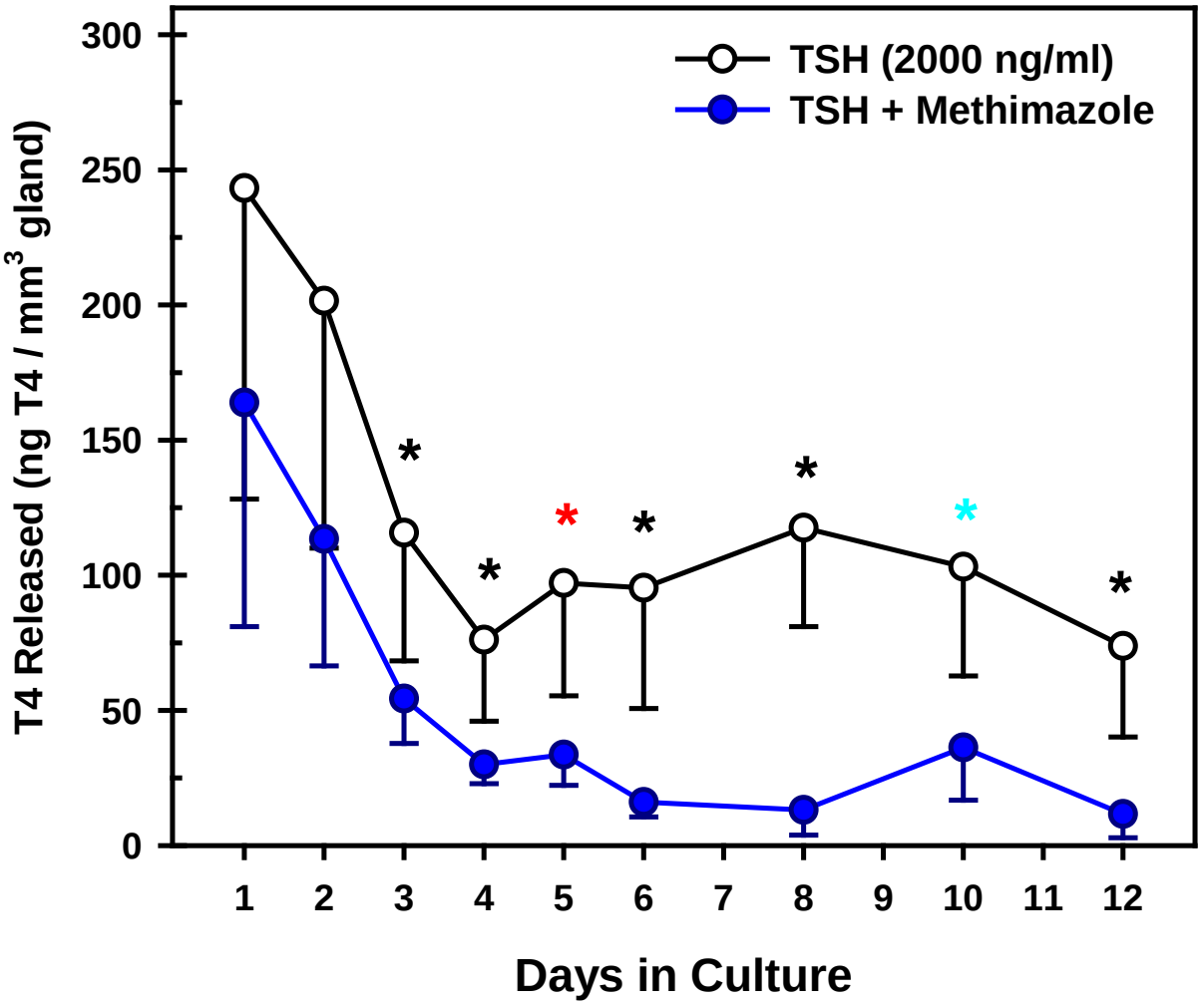
Thyroid Gland Explant Culture:

TSH Dose-Response



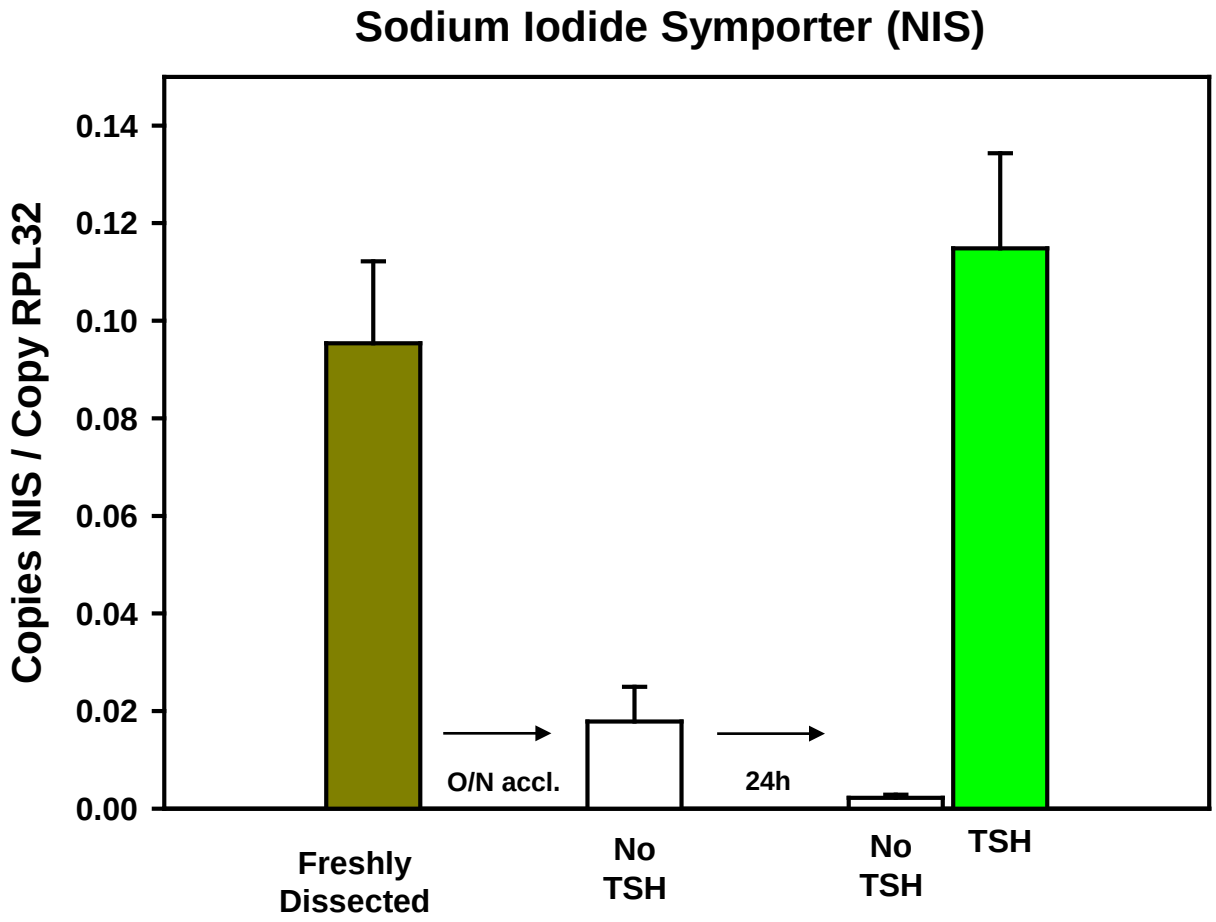
Thyroid Gland Explant Culture:

Time & dose relationship of T4 release inhibition



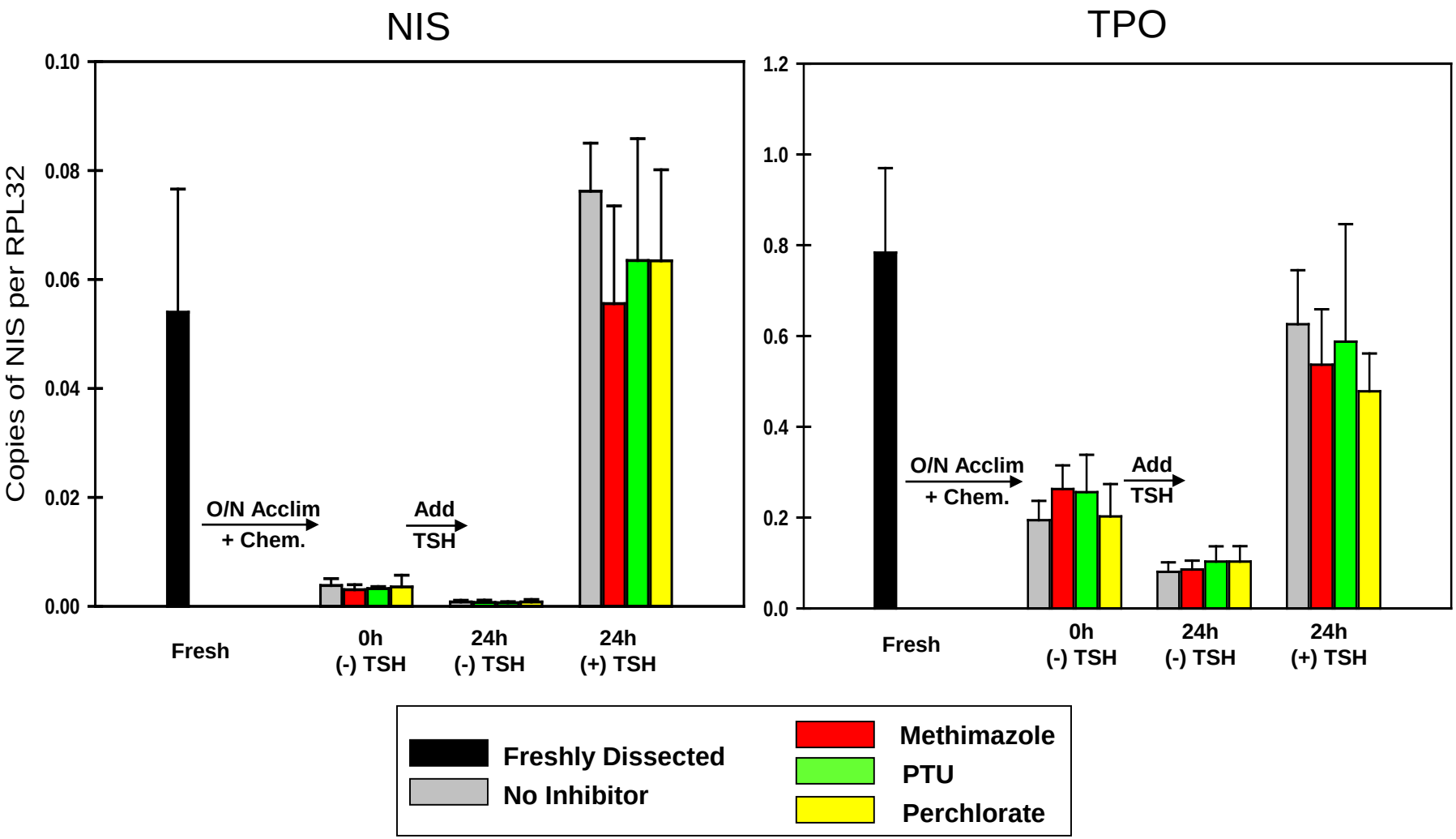
Thyroid Gland Explant Cultures:

Gene Expression Response to TSH

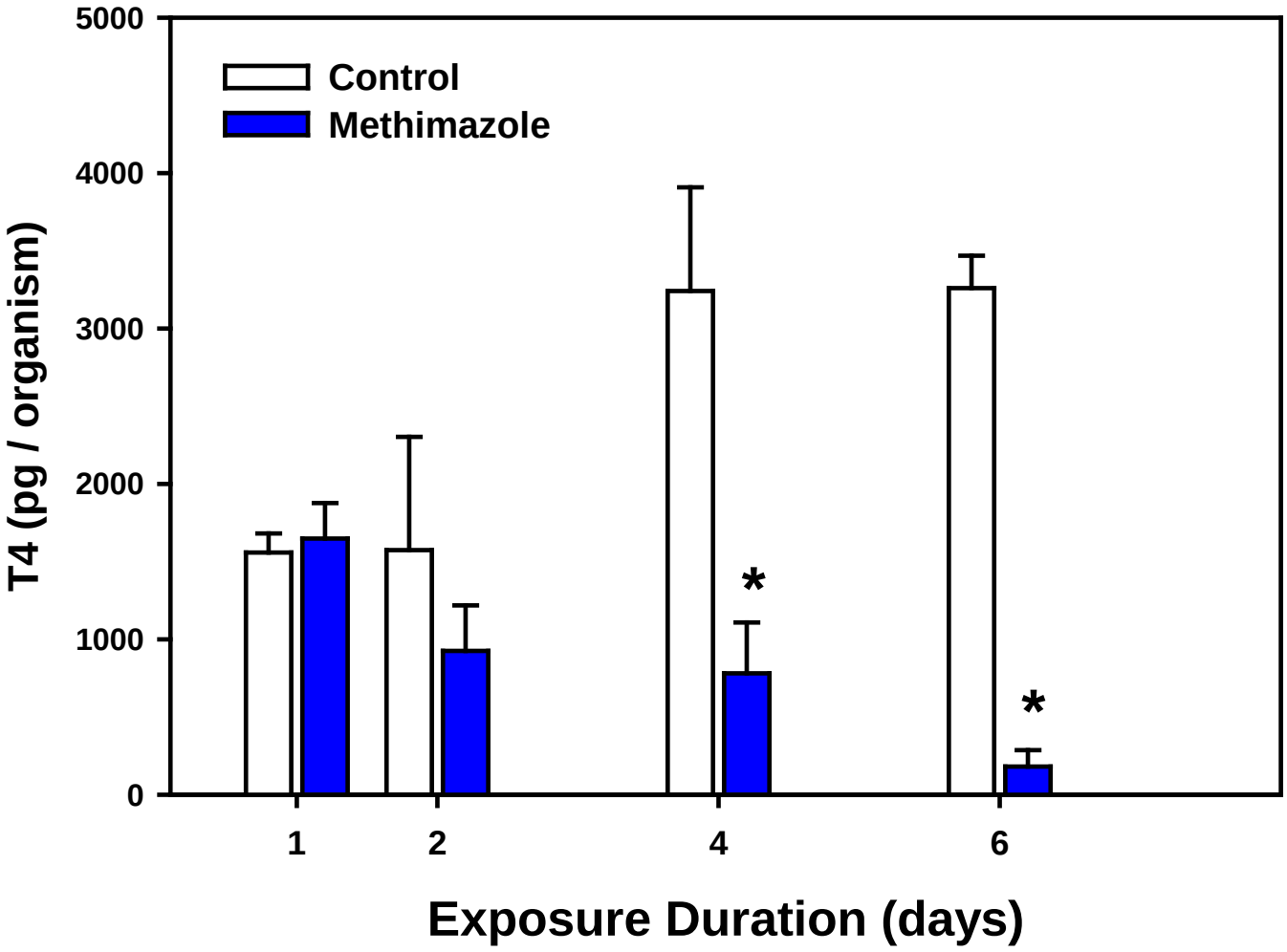


Explant Thyroid Gene Expression:

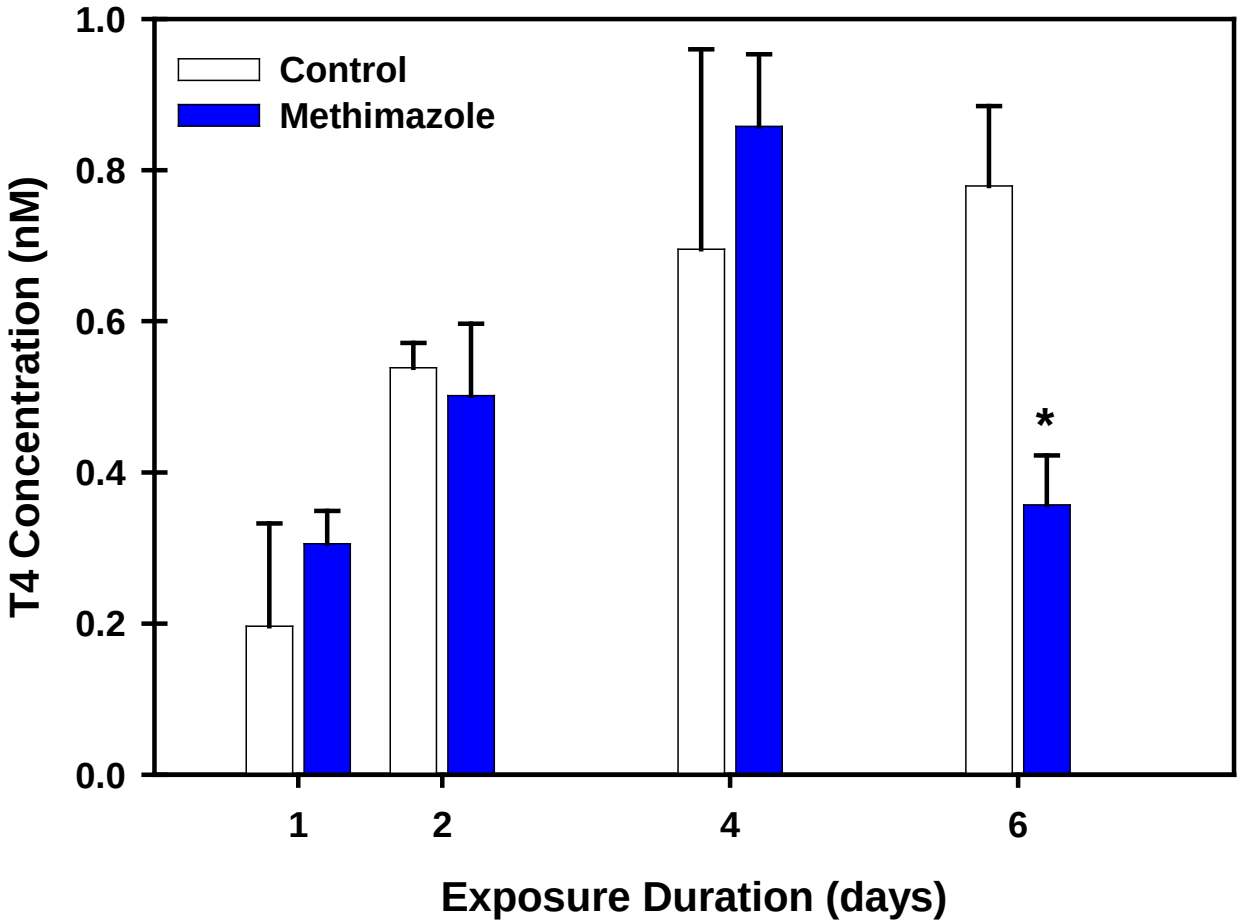
Response to Inhibitors



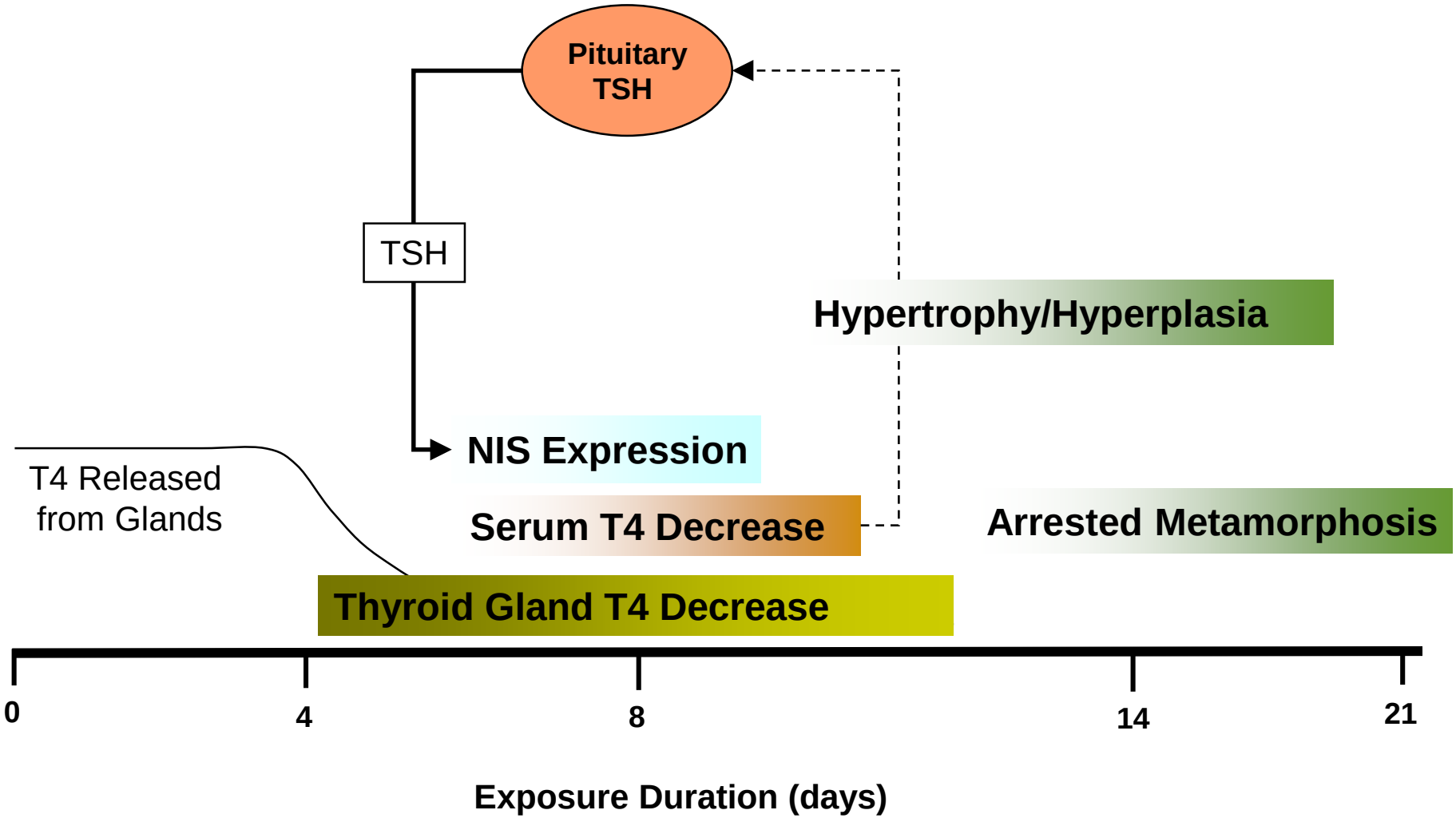
In Vivo Thyroid Gland TH



Serum T4 Following Exposure to Inhibitors



HPT Compensation Summary



Thyroid Explant Culture

Interpretation of compensatory and direct effects

In vitro…

- Release T4 in response to TSH is dose related
- T4 reserves must be depleted before synthesis inhibition significantly affects T4 release

In vivo…

- Early stages are more sensitive to arrested metamorphosis by T4 inhibitors than late stages
- At late prometamorphosis, thyroid glands are larger and reserve T4 is sufficient to complete metamorphosis
- Exposure time 0 does not equal effect time 0 for circulating T4: NEED TO UNDERSTAND DOSIMETRY / PK & PD
- Need to measure circulating hormone levels (T4, TSH) to interpret gene expression and protein responses in vivo

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Thyroid Gland Cell Number:
Normal Development